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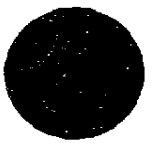
Exposure

ORIGINAL INVESTIGATION



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A further study of FTC yield and nicotine absorption in smokers

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Abstract The relationship between nicotine yield as determined by the FTC method and nicotine absorption was examined in 72 smokers in a more rigorous repetition of a previous study of 33 smokers. For this study, 113 smokers evenly distributed across four FTC "tar" yield ranges were recruited; only 72 demonstrated reasonable compliance with the study criteria with regard to sample collections and cigarette brand style consistency. Subjects recorded the number of cigarettes smoked daily and collected a 24-h urine sample and a saliva sample on 3 consecutive days. Nicotine absorption was determined by monitoring urinary excretion of nicotine and its metabolites. In addition, saliva samples were monitored for cotinine using radioimmunoassay (RIA). The correlation of the relationship for nicotine absorbed per cigarette was positive and significant ($r = 0.31$, $P = 0.008$) but weaker than in the previous study. Only smokers in the highest yield range showed any statistical difference from smokers in the lower ranges. Our results suggest that FTC nicotine yield is weakly related to nicotine absorption and that smoker-controlled factors exert a great influence on the amount of nicotine absorbed by smokers. Compensation is substantial but incomplete for the minority (by market share) of smokers at the low end of the yield scale. It is uncertain how well any alternative set of machine parameters would predict nicotine absorption for the majority of smokers, even if it were more predictive for the small number of smokers at the lower yield part of the range.

Key words Nicotine · FTC smoking · Compensation · Metabolites · Urine · Saliva

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Introduction

Relating the amount of nicotine yielded by cigarettes during machine smoking and the amount absorbed by smokers has been of interest to researchers for most of this century. As early as the 1920s, investigations were done on the amount of nicotine in tobacco smoke from various products on the market (Pfyl and Schmitt 1927). Analytical results were often inconsistent, however, due in part to different parameters for smoke generation used by the various researchers (Bradford et al. 1936). A standardized machine smoking method was proposed that would permit different analysts to rate the quality of tobacco products and also to evaluate various analytical methods being developed for nicotine (Pfyl 1933). Yet as late as 1960, no standard method had been established in the United States. Instead, each tobacco company used its own internal procedure for evaluating products. A compromise of various procedures in use at the time was selected by a committee of the Tobacco Chemists' Conference and tested (Ogg 1964). Interestingly, some parameters were similar to those suggested by Pfyl and Schmitt in 1927 who had observed 15 cigarette smokers and concluded that the average smoker took a 2-s puff of approximately 40 cc twice per minute. With some modifications (Pillsbury et al. 1969), a well-defined regimen (one 35-cc puff of 2 s duration per minute) was adopted in the United States by the Federal Trade Commission (FTC) in 1967 and is now commonly known as "the FTC Method".

The FTC Method originated in product comparison and it was "not to determine the amount of tar and nicotine inhaled by any smoker" (FTC 1967); nonetheless, by 1985 Gori and Lynch indicated that the belief had been widely assumed that it also predicted smoke absorption by individual smokers. Since the observations of Pfyl and Schmitt (1927) were based on human behavior, this belief is understandable. In fact, Bradford et al. (1936) remarked that a successful method should approximate human conditions. But no

reliable analytical methods were in place to determine smoke absorption by smokers. And so, the relationship between smoking machine yields and actual smoke absorption was unclear. As nicotine metabolism became better defined (Benowitz 1991; Kyerematen and Vessell 1991; Byrd et al. 1992; Caldwell et al. 1992; Benowitz et al. 1994) and methods for determining exposure to nicotine (and thus smoke) were developed, numerous studies investigated the relationship between machine-determined nicotine yields and biomarkers of nicotine absorbed by smokers.

These studies led to some general conclusions regarding smoking behavior. It became apparent that a wide range of smoking behaviors was possible for subjects smoking the same type of cigarette, and that some smokers could substantially increase the actual smoke yield of their cigarette relative to that predicted by the FTC method. The latter process is referred to as "compensation". According to the compensation hypothesis, smokers switching to lower FTC yield cigarettes smoke these cigarettes with increased effort (larger, more frequent puffs, deeper inhalation, etc.) to make up, or compensate, for the lower yield and obtain the amount and quality of smoke to which they are accustomed.

Compensatory smoking is a general issue in understanding smoking behavior. A 1986 review of tobacco smoking by the International Agency for Research on Cancer (IARC) concluded that some amount of compensation occurred when smokers switched to lower-yield cigarettes. In 1994, concern over the prevalence of compensatory smoking was raised at a public discussion of the FTC method (National Cancer Institute Conference 1994). Although all researchers recognize that compensation occurs, the degree and duration to which smokers alter smoking behavior (and thus their intake of smoke components) with changes in the "tar" and nicotine yields of cigarettes is unclear. Further, the relative importance of "tar" versus nicotine (or other smoke constituents) in these alterations is uncertain (Rose et al. 1985; Adlkofer et al. 1989; Fairweather 1989; Jarvis 1989; Baldinger et al. 1993, 1995; Hasenfratz et al. 1993; Pritchard et al. 1996). Finally, to the degree that nicotine contributes to these changes, the relative importance of sensory, CNS pharmacology, and systemic effects of nicotine remains to be determined (Walker et al. 1996).

In spite of the uncertainty and complexity of factors driving compensation, it is important to assess its prevalence. Since nicotine is specific to tobacco smoke, many studies have been performed to determine levels of compensation by relating machine smoking nicotine yields and various biomarkers of nicotine absorption. A recent compilation of eight literature data sets that related FTC nicotine ratings and blood cotinine concentrations found that the compiled data set suggested partial compensation by smokers on the order of 50% (Pritchard and Robinson 1996).

Our investigations of FTC nicotine yield and nicotine absorption have been based on the excretion of urinary nicotine and its metabolites. This is a fairly new approach for determining nicotine absorption made possible by new analytical methodology (McManus et al. 1990; Byrd et al. 1992) where the sum of urinary nicotine and its metabolites serves as a biomarker for smoke absorption. The method is believed to account for more than 90% of nicotine absorbed and, since urine and not blood collection is involved, it is relatively non-invasive and permits subjects to smoke ad libitum in their normal environment.

A previous report released from this laboratory (Byrd et al. 1995) addressed the issue of FTC nicotine yield versus measured nicotine absorption in smokers. Using all data available at the time, the report was a compilation of four separate studies, each determining nicotine absorption in a different FTC "tar" yield range and having a total of 33 subjects. Although the individual studies varied slightly in analytical methodology, they all determined nicotine absorption by monitoring nicotine and its metabolites excreted in 24-h urine samples from subjects smoking their self-selected brands ad libitum during a period where the number of cigarettes smoked was recorded. The data showed large individual variability in nicotine absorption within each group and, compared with the literature, a high correlation between FTC nicotine yield and nicotine absorption measured as either mg nicotine per 24-h period ($r = 0.68$) or per cigarette smoked ($r = 0.80$). The shortcomings of that work were 1) insufficient number of subjects to adequately define the relationship and 2) inconsistent sample collection and analytical schemes among the four subject groups. Thus, the objectives in the present study were to repeat the previous work but increase the number of subjects and use standardized sample collection and analytical protocols for each subject. Our goal was to include at least 100 active smokers who were in compliance with the study design and distributed evenly across four FTC "tar" yield ranges. Protocols for assessing subject compliance were included to increase the integrity of the data.

Materials and methods

Subjects

Subjects were recruited through a local independent marketing research firm (Bellomy Research, Inc., Winston-Salem, N.C., USA) using their database of known smokers in the area, from solicitations of smokers in public places, and by random telephone calls. The recruitment goal was to select at least 25 smokers in each of four categories based on FTC "tar" yields for cigarettes: ≤2.0 mg (2 mg or 2MG), 2.1–6.0 mg (Ultralow "Tar" or ULT), 6.1–11.9 mg (Full Flavor Low "Tar" or FFLT), and ≥12.0 mg (Full Flavor or FF). FTC "tar" yields were based on the most recent Tobacco Institute Testing Laboratory Test Results (1994). Targeted subjects were male Caucasians 21–60 years of age who smoked at least 20 non-menthol cigarettes per day and had been loyal to a particular

brand for at least 6 months. Although subjects were isolated from the study purpose, we wanted to reduce the chances that their behavior would change due to any preconceived idea that would have been associated with their occupation. Thus, persons who worked in media, journalism, marketing, law, advertising, tobacco industry (or its subdivisions), or state or federal government health or legal departments were excluded. Persons who consumed an average of more than two alcoholic beverages per day or were using prescription medication were also excluded. Subjects were also excluded if they regularly used any tobacco or nicotine product other than cigarettes (pipes, snuff, nicotine gum, etc.). All of the subjects who met the requirements were found in the Winston-Salem area except for the 2MG smokers. Due to the very small market share of these types of cigarettes (<2% for the two most popular brands in this category; Maxwell Report 1994), insufficient numbers of 2MG smokers were found locally and recruiters went to the cities of Charlotte and Wilmington, N.C., USA, to meet the established quota.

Subjects gave their informed consent prior to participating in the study and all procedures used were approved by a human research review board. The consent form described the procedures to be used and the measurements to be made, but did not disclose the purpose of the tests.

Subjects who participated in the study were re-surveyed approximately 12 months after completion of the study. The questionnaire asked subjects about several consumer products, including the brand and style of cigarette they were smoking and whether they had changed in the last 18 months. These data were compared with the original screening data to assess response consistencies. Subjects who were unavailable for the re-survey were omitted from the final data set. Subjects with inconsistent responses to questions regarding brand loyalty were also excluded. After subject exclusion for the above reasons, 72 subjects were retained in the study.

Scheduling and orientation

The study lasted for a 7-day period. During the study, subjects smoked ad libitum their regular self-selected brand while in their normal home or work environment and returned urine and saliva samples at specific times. Orientation of subjects was performed in the presence of our staff with the exception of the 2 MG smokers (due to the remote site recruitment of these smokers, contracted personnel were used). At orientation sessions, the age, weight, and specific brand were recorded for each subject. Subjects were processed in small groups of 5–15 by three to four staff personnel in a room where smoking was permitted. Some of the staff were smokers as well, which helped put the subjects at ease in talking about their smoking behavior. The subjects were reminded to stay on their regular brand exclusively during the course of the study and to smoke as they normally would. The subjects counted the number of cigarettes smoked each day for 7 days and submitted three consecutive 24-h urine samples and saliva samples on the last 3 days of the study. For their participation, the subjects were financially compensated after the final samples and cigarette counts had been collected.

Counting cigarettes

Subjects were required to maintain a daily record of the number of cigarettes smoked each day for 7 consecutive days, starting on Saturday and ending the following Friday. A log sheet was supplied to facilitate the process.

Collection of urine

Each subject was supplied with two polyethylene 3 l screw-capped specimen bottles (Fisher Scientific, Pittsburgh, Pa., USA). The bottles were stored in a portable cooler (Coleman Personal 16, Coleman Co., Inc., Wichita, Kan., USA) with a reusable ice substitute (Igloo Corporation, Houston, Tex., USA) to retard bacterial growth in the samples. The subjects kept the coolers with them during the 24-h period as they performed their normal routines (work, domestic duties, hobbies, etc.). Urine collection was scheduled on weekdays beginning on Monday when the subjects awoke. Subjects collected their urine for 24 h. Two additional 24-h urine samples were collected on Tuesday and Wednesday. Urine samples were delivered to the laboratory each day after collection was completed and refrigerated until processed later that same day. For processing, total 24-h urine volume and pH were measured for each urine sample and a 10-ml aliquot was frozen at −20°C. A 1-ml sample was taken from the aliquot for nicotine uptake determination and the remainder was submitted for creatinine analysis (Forsyth Laboratories, Winston-Salem, N.C., USA).

Collection of saliva

Three saliva samples were taken from each subject, one on each day of urine collection. The subjects were supplied with sterile Salivette tubes (Sarstedt, Inc., Newton, N.C., USA) and instructed to chew for 1 min on the cotton prior the last meal of the day to synchronize collection of saliva to a time when saliva cotinine was likely to be at steady state.

Analyses of samples

Urine samples were analyzed by a reported thermospray-LC/MS method (McManus et al. 1990; Byrd et al. 1992) that accounts for urinary nicotine and eight metabolites (which includes glucuronide conjugates of three analytes). Samples were prepared and analyzed in random order. Prior to LC/MS analysis, all urine samples were treated with β-glucuronidase (Product No. G-7017, type HP-2, Sigma Chemical Co., St Louis, Mo., USA) to free conjugated nicotine and its metabolites using the method of Kazemi-Vala and Curvall (1994). This enzyme is more aggressive than the one described in an earlier work (Byrd et al. 1992), since it contains some sulfatase activity. The percentages of metabolites existing as conjugates were not determined separately in this study. Total nicotine absorption per day was determined by multiplying the sum of the molar concentrations of nicotine and its metabolites by the total urine volume for that day. The amount of nicotine per cigarette was determined by dividing this number by the number of cigarettes smoked the day the urine was collected. Saliva samples were analyzed for cotinine concentration using the radioimmunoassay (RIA) method of Van Vunakis et al. (1987) and reported as ng/ml. This number was divided by the number of cigarettes smoked on the day the saliva was collected and reported as ng/ml per cigarette. It should be noted that cross-reactivity of RIA polyclonal antibodies with other nicotine metabolites must be considered when assessing the analytical error of these data

Results

Recruitment and sample collection

A total of 113 subjects was recruited for this study. Samples were collected daily on 3 consecutive days resulting in a total of 339 samples of urine and saliva. None of the urine samples were lost, but six samples failed the analysis (excessive turbidity or significant interference for a metabolite). Four saliva samples were lost by the subjects.

Table 1 Average nicotine absorption data for FTC yield ranges in this study. Data for "Cig/24 h", "Measured nicotine", and "Saliva cotinine" are listed as the mean ± standard deviation. The column "Nic ratio" is the measured nicotine absorbance per cigarette relative to that predicted by the FTC method

Group	Age	Wt (kg)	FTC (mg/cig)		Cig/24 h	Measured nicotine		Saliva cotinine		Nic ratio Abs/FTC
			"tar"	Nic		(mg/24 h)	(mg/cig)	(ng/ml)	(ng/ml/cig)	
2MG (n = 23)	43 ± 12	81 ± 13	1.3 ± 0.4	0.14 ± 0.05	37 ± 12	22.2 ± 8.8	0.65 ± 0.30	425 ± 174	12.5 ± 6.7	4.8 ± 2.0
ULT (n = 19)	38 ± 8	83 ± 16	4.9 ± 0.4	0.47 ± 0.06	35 ± 8	21.9 ± 7.8	0.63 ± 0.24	413 ± 170	12.2 ± 5.7	1.4 ± 0.5
FFLT (n = 14)	32 ± 9	82 ± 13	10.1 ± 0.9	0.76 ± 0.05	30 ± 6	20.0 ± 8.6	0.67 ± 0.22	412 ± 209	13.7 ± 5.1	0.9 ± 0.3
FF (n = 16)	33 ± 8	82 ± 15	16.5 ± 0.8	1.20 ± 0.14	32 ± 6	27.9 ± 9.6	0.89 ± 0.30	526 ± 201	16.8 ± 6.6	0.8 ± 0.3

Compliance and final selection of subjects

Compliance was an important part of this study since, even though the participants were paid, behavior could not be monitored. Complete collection of urine was a particular concern, since performing this task rigorously for 3 consecutive days required diligence. Incomplete collection or dilution with another liquid would impact the results since the determined concentration in the urine is multiplied by the total 24-h volume to determine daily nicotine absorption. Urine volume can vary a great deal for a given individual; however, creatinine excretion remains fairly constant. Normal creatinine excretion for a 70 kg adult male is approximately 1–2 g/24 h with between-day variability of 15–20% (Tietz 1987). Creatinine measurements were used to assess sample collection compliance using the following protocol. Samples with daily creatinine excretion <0.75 g were rejected. Samples with relative standard deviations >50% (coefficient of variation of 0.5) over three collections had the urine sample with the lowest daily amount of creatinine rejected. This resulted in the rejection of 21 samples in the first case and four samples in the second case out of 339 total samples resulting in a net sample count of 314 urine samples. The rejected samples accounted for less than 8% of the total number of collected samples.

The urine sample set can be summarized as follows: of the 113 subjects, 88 had data on a complete set of three urine samples, 19 had one sample missing, and six had two samples missing. All days were averaged for each subject in the final data set.

Compliance with regard to truthfulness of brand and numbers of cigarettes smoked per day was also a concern in this study. The numbers reported here depend a great deal on the fidelity of the subjects in these matters; however, a secondary questionnaire in the form of a general consumer database update was given a year later to as many subjects as could be located. Part of the survey dealt specifically with the brand and type of cigarette smoked and any changes made in the past 12–18 months. The purpose of this survey was to identify any misclassified smokers. Of the 113 subjects in the study, 89 (79%) were located and surveyed. Of these 89 subjects, 59 (66%) gave answers consistent with their initial survey and reported no changes. A total of 12 of the 89 (13%) had switched from their initial brand, presumably after the study (subjects who switched after the study could not be distinguished from those who may have switched just before the study). One subject had quit smoking since his participation. Inconsistent answers were given by 17 of the 89 subjects (19%) where the subjects reported smoking a completely different brand or type of cigarette than initially reported and had not switched over the last 18 months. The data from this last set of subjects were rejected. Thus, the final sample set consisted of 72 subjects. The distribution of the final sample set slightly over-represented smokers in the 2MG and ULT groups. Average data for each "tar" group are listed in Table 1.

Correlations of FTC nicotine yield and nicotine absorption parameters

Nicotine absorption was correlated with FTC nicotine yield for both individual subjects and by the FTC "tar"

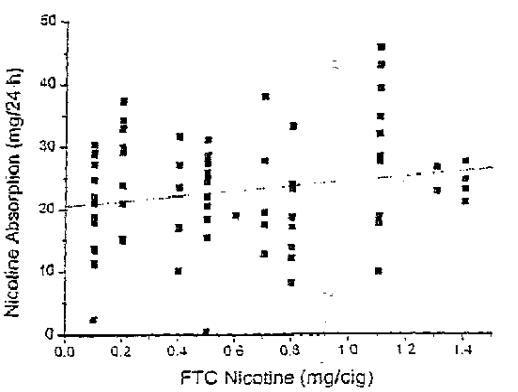

Fig. 1 Plot of FTC nicotine yield (mg/cig) versus nicotine absorption (mg/24 h) for individual subjects. The linear regression equation is: $Y = 4.1X + 20.6$, $r = 0.18605$, $P = 0.11764$

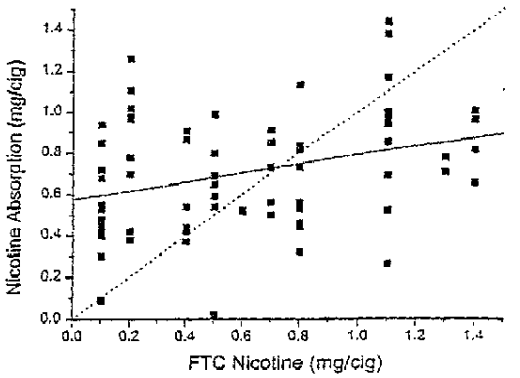

Fig. 2 Plot of FTC nicotine yield (mg/cig) versus nicotine absorption (mg/cig) for individual subjects. The linear regression equation is: $Y = 0.214X + 0.576$, $r = 0.30869$, $P = 0.00833$. The *dotted line* indicates the line of unity response for FTC predicted absorption of nicotine

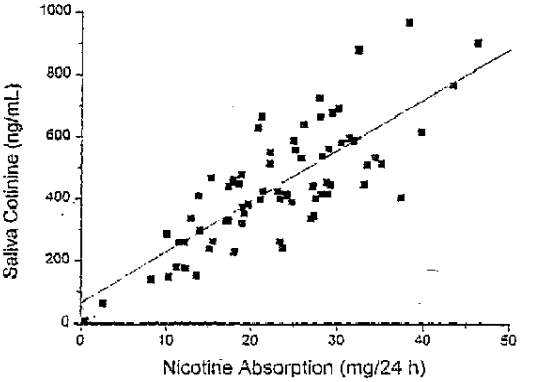

Fig. 3 Plot of nicotine absorption (mg/24 h) versus saliva cotinine (ng/ml) for individual subjects. The linear regression equation is: $Y = 16.4X + 64.9$, $r = 0.78154$, $P = 5.45 \times 10^{-16}$

groups described above (using the average FTC nicotine yield for that group). The four absorption parameters correlated were (as listed in Table 1): mg nicotine/24 h, mg nicotine/cigarette, ng cotinine/ml of saliva, and ng cotinine/ml of saliva /cigarette. The last column in Table 1 is the ratio of measured nicotine absorbance per cigarette relative to that predicted by the FTC method. The average relative standard deviation for all 72 subjects was 20% for mg nicotine/24 h, and 21% for the three other parameters listed in Table 1. The nicotine absorption parameters are plotted for individual smokers in Figs 1 and 2. Total nicotine absorption as mg/24 h for each subject (Fig. 1) shows that the correlation was positive, but small ($r = 0.19$) and not significantly different from zero ($P > 0.05$). Figure 2 presents the same data but corrected for the number of cigarettes smoked per day. The correlation was higher ($R = 0.31$) and significant ($P < 0.05$). Saliva cotinine showed similar results with the absolute concentration having a weak and insignificant correlation with FTC nicotine ($r = 0.15$, $P = 0.212$) but higher and significant correlation when corrected for the number of cigarettes smoked ($R = 0.244$, $P = 0.039$). The weaker correlations in the present study are driven by relatively higher nicotine absorption for the lower yield smokers and also by relatively lower nicotine absorption for the higher yield smokers compared to the previous study (Byrd et al. 1995).

Table 1 lists averaged data for each of the four groups. Though correlations for averaged data were positive, none were significant at $P = 0.05$. A Duncan's multiple range test was used to compare group means. With $P = 0.05$, a comparison of daily total nicotine absorption (mg/day) showed that only the FFLT group was different from the FF group. For nicotine absorption per cigarette, all three lower yield groups were different from the FF group but not different from each other. There were no differences in saliva cotinine (ng/ml) among the groups; however, correcting for numbers of cigarettes smoked (ng/ml/cigarette) showed a difference in the ULT and FF smokers. Other trends noted in Table 1 are similar body weights across the groups, but numbers of cigarettes smoked per day and age tended to increase slightly with decreasing FTC "tar" and nicotine yields.

Nicotine absorption (mg/24 h) was correlated with the three major urinary metabolites (mg nicotine equivalents/24 h) and also with saliva cotinine (ng/ml). The correlation coefficients for urinary *trans*-3′-hydroxycotinine, cotinine, and nicotine were 0.85, 0.83, and 0.66, respectively, with $P < 0.001$. Saliva cotinine is plotted against nicotine absorption in Fig. 3 and shows the correlation of 0.78 between these two parameters.

Metabolite distribution

The average distribution of nicotine and individual metabolites relative to the total sum is listed in Table 2 for this study and is compared with three previous studies. The profile is in good agreement with literature data, with each distribution having a large standard deviation for all metabolites. Nicotine-N′-oxide (NNO) averaged considerably higher than the literature values (12.2% compared to 5.5% average of the three literature means). This may result from the more aggressive enzyme treatment used in this study that could have released additional NNO from an unknown conjugate or produced a co-eluting interference.

Estimation of daily nicotine absorption from saliva cotinine

Saliva cotinine concentrations were used to estimate daily nicotine absorption for each smoker. This amount was compared to the amount determined by urinary

Table 2 Urinary excretion as molar percentage of total recovered nicotine and metabolites: average ± standard deviation for each FTC "tar" category. *3HC* trans-3'-hydroxycotinine, *COT* cotinine, *NIC* nicotine, *NNO* nicotine-*N'*-oxide, *CNO* cotinine-*N*-oxide, *DMC* demethylcotinine (norcotinine)

GROUP	3HC	COT	NIC	NNO	CNO	DMC
This study	43.1 ± 12.2%	25.4 ± 7.1%	14.4 ± 6.8%	12.2 ± 8.5%	3.8 ± 1.4%	1.1 ± 0.7%
Byrd et al. (1992)	45.3 ± 10.4%	29.8 ± 7.8%	12.7 ± 3.6%	6.7 ± 3.0%	4.1 ± 2.1%	1.4 ± 0.4%
Benowitz et al. (1994)[a]	46.9 ± 18.4%	29.1 ± 10.9%	15.0 ± 7.3%	3.7 ± 0.9%	4.5 ± 1.5%	n.d.
Byrd et al. (1995)	53.3 ± 15.6%	26.6 ± 10.8%	10.4 ± 3.9%	6.0 ± 2.6%	3.1 ± 0.7%	0.7 ± 0.5%

[a]Percentages based on summation of free and conjugated materials for 3HC, COT, and NIC; standard deviations were summed. Nornicotine was also determined for these samples as 0.65% ± 0.15

excretion of nicotine and its metabolites. Using the ratio of saliva to plasma cotinine concentrations determined by Curvall et al. (1990a) (1:1.27) and the equation of Benowitz and Jacob (1994) to determine daily nicotine absorption from steady-state plasma cotinine concentrations, the following equation was produced:

$$\text{Nic. absorption (mg/24-h)} = K \times \text{Sal. Cot. (ng/ml)}/1.27$$

where K is the conversion factor to convert the time-weighted average plasma cotinine concentration (ng/ml) to daily intake of nicotine (mg/24 h). Benowitz and Jacob found that K was 0.08 on average in their study and ranged from 0.047 to 0.102 ($n = 13$). Using their average value for K, we determined the estimated daily nicotine absorption for each subject in this study ($n = 72$) and compared it to the amount determined by urinary excretion of nicotine and its metabolites. The saliva cotinine estimate of nicotine absorption averaged 1.24 ± 0.33 of the urinary excretion amount. Solving the equation above for K, we determined an average K of 0.07 ± 0.02 with a range of 0.040–0.124; however, since our method assumes more than 90% recovery of total nicotine absorbed, our determined K value might be as much as 10% lower than the literature value.

Discussion

The measurement of urinary nicotine and its metabolites in 72 smokers who complied with the study protocol was used to define their nicotine absorption across FTC "tar" yield categories. The determination of saliva cotinine served as a secondary biomarker for nicotine absorption and correlated well with urinary excretion data. Results of the previous study (Byrd et al. 1995) were markedly different from other studies in the literature. Relative to the present study, this difference was caused by relatively lower nicotine absorption by smokers of the lower yield cigarettes and also higher nicotine absorption by smokers of the higher yield cigarettes in the Byrd et al. (1995) study. The higher absorption by the lower group in the present study may be accounted for, in part, by the higher range used for the lowest group in this study (0–2 mg "tar") versus the previous study (0–1 mg "tar").

By starting with over 100 subjects, we were able to obtain a large sample size which met high standards for compliance and sample integrity. We believe the present study therefore more accurately reflects nicotine absorption by smokers smoking cigarettes in the categories tested than in the earlier study. More subjects were tested and they were more evenly distributed across the four FTC "tar" ranges chosen. In addition, all the samples were collected under the same protocol and analyzed together, keeping the sampling and analytical parameters as uniform as possible. While it was surprising that the correlation between nicotine absorption and FTC nicotine yield would be so different from the previous study, the results were more representative of data reported in the literature (Pritchard and Robinson 1996b).

The subjects were limited to male Caucasian smokers of 20 or more non-menthol cigarettes per day. This was done to avoid possibly confounding factors of gender, race, and mentholation. Thus, these data reflect only a subset of the general smoking population. It is unknown if smokers of fewer cigarettes per day or mentholated cigarettes, female smokers, or members of other races absorb the same amount of nicotine per cigarette as reported here.

Considerable effort was directed toward subject compliance with the study protocol by making the subjects comfortable with their participation without revealing the purpose of the study. One concern was that health risks associated with smoking would cause smokers to report lower numbers of cigarettes than actually smoked (Lee 1988). By making each group comfortable enough so that they felt free to smoke during explanation of the study, we hoped to increase the probability that the subjects would truthfully report the numbers of cigarettes smoked and also collect all of their urine samples as required. To check for compliance with urine collection, creatinine measurements were monitored for daily variations in urine output that might reflect incomplete capture of urine or dilution with other fluids. Identified samples were rejected according to the criteria described in the Results section. Also, compensation was contingent upon completion of the study (after all samples and record sheets were collected) and thus served as an additional incentive

for the subjects to maintain truthful reporting. Finally, re-surveying the subjects 1 year after the study to confirm their brand and style of cigarette listed in the initial recruitment increased confidence that these data accurately reflect a subject's usual brand. All these measures were employed to improve the integrity of the data; however, whether or not absolute compliance was maintained by these subjects for all parameters, particularly numbers of cigarettes smoked per day and fidelity to their stated brand and style, is unknown. Misclassification of smoking status (smoker versus nonsmoker) has been explored (Coultas et al. 1988; Lee 1988; Perez-Stable et al. 1992; Lee and Forrey 1995), but misclassification of cigarette brand and style among smokers has had only limited study (Cohen 1996). The apparent brand and style misclassification by 19% of resurveyed subjects demonstrates the problems associated with this type of study.

Recovery of high percentages of nicotine and its metabolites in urine offers the best means to accurately assess human exposure to nicotine (Benowitz 1991; Byrd et al. 1992; Benowitz et al. 1994). Renal clearance of nicotine and its metabolites depends upon urinary pH and flow (Matsukura et al. 1979; Rosenberg et al. 1980). These factors are influenced by physical exercise, posture, and diet in the individual. These all may vary from time to time for an individual and thus give rise to daily fluctuations in nicotine disposition. For this reason, three consecutive 24-h urine samples were taken from the subjects in this study to minimize effects of these parameters and provide a good representative measurement of nicotine absorption. The day-to-day variability of nicotine absorption for the 72 subjects averaged 20%.

With the exception of the minor metabolite NNO, the distributions of urinary metabolites were similar to those reported in the literature (see Table 2). The relative amount of NNO was approximately twice as high in this study as previously reported values and may be related to use of a more aggressive enzyme treatment that has sulfatase activity. Since NNO is a relatively minor metabolite and all urine samples were treated with the same enzyme, this would have little impact on the total nicotine absorption or subsequent correlations.

By including saliva cotinine measurements, our study helps better define the distribution of nicotine in humans. Plasma cotinine is known to be directly related to nicotine absorption (Galeazzi et al. 1985) and saliva cotinine has been reported to describe cotinine disposition in the body as well as plasma concentrations (Curvall et al. 1990a). Saliva cotinine is indicative of exposure to tobacco smoke but the dose-response relationship is unclear (Schepers and Walk 1988; Etzel 1990; Swan et al. 1993). While high correlation between plasma and saliva cotinine has been demonstrated (Curvall and Enzell 1986; Curvall et al. 1990b; Schramm et al. 1992), our study is the first to correlate the sum of urinary nicotine and its metabolites and saliva cotinine for samples from the same set of subjects taken at the same time. The correlation ($r = 0.78$) between nicotine absorption (mg/24 h) and saliva cotinine (ng/ml) in our study and the similarity of conversion factor K determined from our data and that reported by Benowitz and Jacob (1994) suggests that saliva cotinine can provide an estimate of nicotine absorption in smokers; however, the inter-individual variability which showed a three-fold range in conversion factor K suggests that the use of saliva cotinine as a quantitative biomarker is limited. With regard to the methods employed in our study, this relatively high correlation enhances the validity of the data since these two measurements were obtained in different fluids and used completely different analytical techniques.

As in the previous study, wide variability of nicotine absorption by smokers occurred within each category. Reports of wide inter-subject variability with regard to nicotine absorption biomarkers are fairly common in the literature (Benowitz et al. 1983, 1994; Hill et al. 1983; Wall et al. 1988; Byrd et al. 1992) and point to large differences in inhalation behaviors and nicotine metabolism. Inter-subject variability is an important factor in the weak correlations between FTC machine yields and nicotine absorption in our data and those of others.

Those smokers showing the most deviation from FTC yields, the 2MG group, were also the most difficult to recruit as they comprise <2% of market share (based on the two most popular brands; Maxwell Report 1994). The large changes in smoking behaviors (number of puffs, puff interval, puff duration, etc.) presumably required to achieve greater yields of smoke constituents from these cigarettes do not seem to be preferred by most smokers. In fact, only 12.2% of the US smoking population smoked cigarettes in the 0–6 mg range (Maxwell Report 1994). This category corresponds to the 2MG and ULT smokers in this study. The data in Table 1 show that the remaining smokers (FFLT and FF representing 87.8% of the US market share) absorbed, on average, within 25% of the FTC predicted nicotine value (mg/cigarette) in their respective categories. Since most smokers absorb on average approximately 1 mg nicotine per cigarette (0.67 for FFLT and 0.89 for FF smokers in this study), it is not surprising that the FTC method is valid for these smokers. However, the current FTC method is not a valid predictor over the entire range of cigarettes on the market. This is the dilemma of finding a universal set of smoking parameters; it is uncertain how well a new set of parameters would predict nicotine absorption for the majority smokers even if it were more predictive for the small number of smokers at the lower yield part of the range.

In the previous study we observed that the correlation between nicotine absorption and FTC nicotine yield was positive, significant ($P < 0.05$), and strong

($r > 0.5$). When we tested this hypothesis in a larger sample, we found that the correlation was positive and significant, but weak. In fact, there were no differences between nicotine absorption and FTC nicotine yield among the three lower categories in this study. An alternative hypothesis is that smoker-controlled parameters such as puff volume and duration, puff frequency, fraction of smoke inhaled, and vent blocking exert a greater influence on the amount of nicotine absorbed by smokers than cigarette design parameters. For example, blocking air dilution holes on low "tar" cigarettes has been shown to increase the nicotine yield relative to the FTC method (Kozlowski et al. 1982; Höfer et al. 1991). Our data show that compensation occurs but is not complete for smokers of the lowest yield cigarettes. We conclude that it is difficult (requires more effort), but not impossible, for some smokers of the lowest yield cigarettes to obtain smoke amounts similar to those of higher yield cigarettes.

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